Supplementary Material

Table 8. Strains and primers used in this study

Strains	Description	Generation method	Source (reference)
E. coli			
BW25113	wildtype strain (*lacI*^{q} *rrnB*$_{T14}$ Δ*lacZ*$_{WJ16}$ *hsdR514* Δ*araBAD*$_{AH33}$ Δ*rhaBAD*$_{LD78}$)		Datsenko and Wanner (2000)
ECA101	Δ*rpoE*	Deletion of *rpoE* in BW25113	This study
ECA199	Δ*rpoE* Φ*rpoEp-rpoErseAB*	pAH125::*rpoEp-rpoErseAB* integration into ECA101	This study
ECA203	Φ*rpoEp-lacZ*	pAH125::*rpoEp* integration into BW25113	This study
ECA204	Δ*copA*	P1 Δ*copA::cat* → BW25113	This study
ECA205	Δ*cusCFBA*	P1 Δ*cusCFBA::cat* → BW25113	This study
ECA206	Δ*cueO::cat*	P1 Δ*cueO::cat* → BW25113	This study
ECA207	Δ*rpoE* Δ*copA*	P1 Δ*copA::cat* → ECA101	This study
ECA208	Δ*rpoE* Δ*cusCFBA*	P1 Δ*cusCFBA::cat* → ECA101	This study
ECA209	Δ*rpoE* Δ*cueO::cat*	P1 Δ*cueO::cat* → ECA101	This study
ECA210	Δ*copA* Δ*cusCFBA*	P1 Δ*cusCFBA::cat* → ECA204	This study
ECA211	Δ*copA* Δ*cueO::cat*	P1 Δ*cueO::cat* → ECA204	This study
ECA212	Δ*cusCFBA* Δ*cueO::cat*	P1 Δ*cueO::cat* → ECA205	This study
ECA213	Δ*rpoE* Δ*copA* Δ*cusCFBA*	P1 Δ*cusCFBA::cat* →ECA207	This study
ECA214	Δ*rpoE* Δ*copA* Δ*cueO::cat*	P1 Δ*cueO::cat* → ECA207	This study
ECA215	Δ*rpoE* Δ*cusCFBA* Δ*cueO::cat*	P1 Δ*cueO::cat* → ECA208	This study
ECA216	Δ*copA* Δ*cusCFBA* Δ*cueO::cat*	P1 Δ*cueO::cat* → ECA210	This study
ECA217	Δ*rpoE* Δ*copA* Δ*cusCFBA* Δ*cueO::cat*	P1 Δ*cueO::cat* → ECA213	This study
ECA219	Φ*cueRp-lacZ*	pAH125::*cueRp* integration into BW25113	This study
ECA220	Δ*rpoE* Φ*cueRp-lacZ*	pAH125::*cueRp* integration into ECA101	This study
ECA222	Φ*cueOp-lacZ*	pAH125::*cueOp* integration into BW25113	This study
ECA223	Δ*rpoE* Φ*cueOp-lacZ*	pAH125::*cueOp* integration into ECA101	This study
ECA225	Φ*copAp-lacZ*	pAH125::*copAp* integration into BW25113	This study
ECA226	Δ*rpoE* Φ*copAp-lacZ*	pAH125::*copAp* integration into ECA101	This study
ECA227	Δ*zntA*	P1 Δ*zntA::cat* → BW25113	This study
ECA228	Δ*zitB*	P1 Δ*zitB::cat* → BW25113	This study
ECA229	Δ*rpoE* Δ*zntA*	P1 Δ*zntA::cat* → ECA101	This study
ECA230	Δ*rpoE* Δ*zitB*	P1 Δ*zitB::cat* → ECA101	This study
ECA231	Δ*zntA* Δ*zitB*	P1 Δ*zitB::cat* → ECA227	This study
ECA232	Δ*rpoE* Δ*zntA* Δ*zitB*	P1 Δ*zitB:cat* → ECA229	This study
Primers	**Sequence (5'-3')**		

FRT-rpoE-Ec-down	TGG TTT GGG GAG ACT TTA CCT CGG ATG AGC GAG CAG TTA GCG ATT GTG TAG GCT GGA GCT
FRT-rpoE-Ec-up	CCC TTA TTC AGT ATC CCG CTA TCG TCA ACG CCT GAT AAG CCA TGG TCC ATA TGA ATA TCC TCC
Ec-rpoEp-Pst	AAA CTG CAG AGA ACG ATG ACC TGA TGC TGG
Ec-rpoEp-Eco	AAA GAA TTC CCA GAC TCG CCA CTT TAT GCT
rseB(Ec)pASK-Pst	AAA CTG CAG TCA TTG CGC TGC CCC GAA
cueRp-Pst	AAA CTG CAG GAT TAT GCT GAT GAC GGC GGC
cueRp-Eco	AAA GAA TTC GCG TCA CCA GCC CCT TCT CTT
cueOp-Pst	AAA CTG CAG TGA GCG AAA AAG ACC AGT GCG
cueOp-Eco	AAA GAA TTC GGC ATC GGT CGT GAC CAA ATC
copAp-Pst	AAA CTG CAG TCG CCA GAA AGG GAA TGT AAT
copAp-Eco	AAA GAA TTC ATA GAC ACA TCC GCC TGC TCA

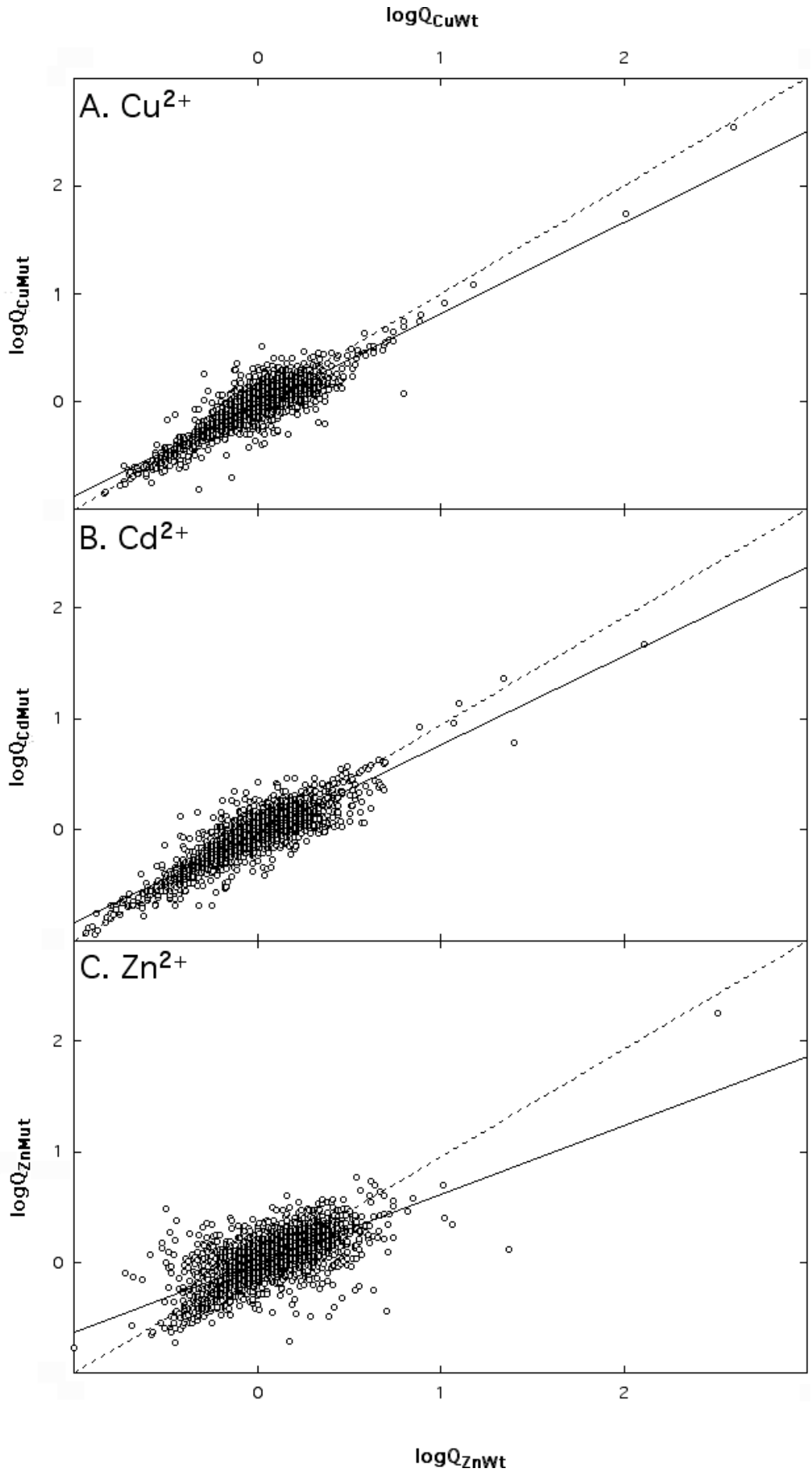
logQCuWt
A. Cu2+
logQCuMut
B. Cd2+
logQCdMut
C. Zn2+
logQZnMut
logQZnWt
0
1
2

Figure 4. Comparison of changes in gene-specific transcript levels in Δ*rpoE* mutant and wild type cells. The ratios of the gene specific transcript levels as determined by microarray experiments were calculated as $Q_{metalWt}$ = transcript level in wild type after metal treatment divided by the transcript levels in untreated wild type cells with "metal" being copper, cadmium or zinc. This was done for 4239 genes. The calculation was repeated for the respective transcript levels in Δ*rpoE* mutant cells. For each metal and gene, the decadic logarithm (basis 10) of the Q-value determined for the mutant cells was plotted against that of the wild type cells.

Minimum information about a microarray experiment - MIAME
Draft March 21, 2001 (based on November 17, 2000) updated 30 July 2001.

MIAME compliant experiment

1. Experimental design: the set of the hybridisation experiments as a whole
This section gives information describing the experiment, which may consist of one or more hybridisations, as a whole. Normally 'experiment' should include a set of hybridisations which are inter-related and performed in a limited period of time. For instance, it may be all the hybridisations related to research published in a single paper.
author (submitter), laboratory, contact information, links (URL) -

a) author (submitter), laboratory, contact information, links (URL), citation

Cornelia Große
Institute of Microbiology, University of Halle-Wittenberg, Kurt-Mothes-Str.3,
D-06120 Halle, Germany

b) type of the experiment - maximum one line

mutant vs. wild type

c) experimental variables, i.e. parameters or conditions tested (e.g., time, dose, genetic variation, response to a treatment or compound)

response to compound - metals: $ZnCl_2$, $CdCl_2$, $CuCl_2$

d) single or multiple hybridisations

For multiple hybridisations:

* serial (yes/no)

* grouping(yes/no)

for multiple hybridisations:

* serial (yes/no)

* type (e.g., time course, dose response)

grouping (yes/no)

* type (e.g., normal vs. diseased, multiple tissue comparison)

mutant vs. wild type

Relationships between all the samples, arrays and hybridisations in the experiment: each sample, each array, and each hybridisation should be given a unique ID or number, and all the relationships should be listed, possibly with appropriate comments.

Samples: wild type, mutant

Arrays:

28,29,30,31,32,33,34,35,36,37,38,39,41,42,43,44

Hybridisations:

H1 : Array 28, treated with Zn, mutant Cy5 vs. wild type Cy3

H2 : Array 29, treated with Zn, mutant Cy3 vs. wild type Cy5

H3 : Array 30, treated with Zn, mutant Cy5 vs. wild type Cy3

H4 : Array 31, treated with Zn, mutant Cy3 vs. wild type Cy5

H5 : Array 32, untreated mutant Cy5 vs. wild type Cy3

H6 : Array 33, untreated mutant Cy3 vs. wild type Cy5

H7 : Array 34, untreated mutant Cy5 vs. wild type Cy3

H8 : Array 35, untreated mutant Cy3 vs. wild type Cy5

H9 : Array 36, treated with Cu, mutant Cy5 vs. wild type Cy3

H10: Array 37, treated with Cu, mutant Cy3 vs. wild type Cy5

H11: Array 38, treated with Cu, mutant Cy5 vs. wild type Cy3

H12: Array 39, treated with Cu, mutant Cy3 vs. wild type Cy5

H13: Array 41, treated with Cd, mutant Cy5 vs. wild type Cy3
H14: Array 42, treated with Cd, mutant Cy3 vs. wild type Cy5
H15: Array 43, treated with Cd, mutant Cy5 vs. wild type Cy3
H16: Array 44, treated with Cd, mutant Cy3 vs. wild type Cy5

H1+H3, H2+H4, H5+H7, H6+H8, H9+H11, H10+H12, H13+H15, H14+H16 are biological replicates
H1+H2, H3+H4, H5+H6, H7+H8, H9+H10, H11+H12, H13+H14, H15+H16 are technical replicates (dye swap)
same RNA prep was used for hybridisations for technical replicates
cDNA labelling was done individually for each experiment

e) quality related indicators quality control steps taken:

***** biological replicates

2

***** technical replicates (replicate spots or hybs)

2 for each biological experiment

***** polyA tails

***** low complexity regions

***** unspecific binding

***** other

Oligos from *Arabidopsis thaliana* as negative control

f) optional user defined "qualifier, value, source" list

g) a free text description of the experiment set or a link to a publication

The wild type and mutant *E.coli* (BW25113: $lacI^q$ $rrnB_{T14}$ $\Delta lacZ_{WJ16}$ *hsdR514* $\Delta araBAD_{AH33}$ $\Delta rhaBAD_{LD78}$) derivat of F^- λ^- K12 strain BD792) were grown at 37 °C in Mineral salt medium + 0.2 % Glcerol + 0.3 % casaminoacids.

At a cell turbidity of 100 Klett the different metals (100 μM ZnCl2 or 250 μM CuCl2 or 25 μM CdCl2) were added and incubation was continued for 10 min. Then cells were harvested.

2. Array design: each array used and each element (spot) on the array

There are two parts of this section:
2.1 describes the list of physical arrays themselves, each
of these referring to specific array design types described in 2.2. We expect that the array design type
descriptions will be given by the array providers and manufactures, in which case the users will simply
need to reference them.
2.2 Array design This section consists of three parts
a) description of the array as the whole,
b) description of each type of elements (spot) used (properties that are typically common to many elements
(e.g., 'synthesized oligo-nucleotides' or 'PCR products from cDNA clones'),
c) description of the specific properties of each element, such as the DNA sequence.
(In practice, the last part will be provided as a spread-sheet or tab-delimited file)

2.1 Array copy: each array used and each element (spot) on the array.

***** unique id as used in part 1

sl28 - 44 except of No.40

***** array design name (e.g Stanford human 10K set)
(for commercial or standard arrays a unique ID given by the provider may be used)

MWG *E.coli* K12 V2 Array

2.2

a) array related information

* array design name (e.g., "Stanford Human 10K set")

MWG *E.coli* K12 V2 Array

* platform type: in situ synthesized or spotted

spotted

* array provider (source)

in-house (MWG Biotech, Ebersberg, Germany)

* surface type: glass, membrane, other

glass

* surface type name

in-house (MWG Biotech, Ebersberg, Germany)

* physical dimensions of array support (e.g. slide)

75 x 25 mm

* number of elements on the array

4608

* a reference system allowing to locate each element (spot) on the array (in the simplest case the number of columns and rows is sufficient)

by coordinate, referencing to an external data table,
16 subgrids
ordered 4 x 4
counted left right, top down, 16 rows, 18 columns per subgrid,

* production date

030303

* production protocol (obligatory if applicable)

* optional "qualifier, value, source" list (see Introduction)

b)properties of each group of elements (spots) on the array;
elements may be simple, i.e., containing only identical molecules, or composite, i.e., containing different oligonucleotides obtained from the same reference molecule;

* element type id

* simple or composite

simple

* element type: synthesized oligo-nucleotides, PCR products, plasmids, colonies, other

synthesized oligo-nucleotides

* single or double stranded

single

* element (spot) dimensions (approximate diameter)

8-24 μm

* element generation protocol that includes sufficient information to reproduce the element

MWG protocol

* attachment (covalent/ionic/other)

covalent

* optional "qualifier, value, source" list (see Introduction)

c) specific properties of each spot on the array:

* element type ID from 2.2b

synthesized oligo-nucleotides

* position on the array allowing spot identification in the image (see 5a below)

available on request

* clone information, obligatory for elements obtained from clones:
(clone ID,clone provider,date,availability)

* sequence or PCR primer information:
(sequence accession number in DDBJ/EMBL/GenBank if known, sequence itself (if databases do not contain it, primer pair information, if relevant)

MWG protocol, NCBI/Genebank NC00913, U00096.1

* for composite oligonucleotide elements:
(oligonucleotide sequences if given,given number of oligonucleotides and the reference sequence (or accession number), otherwise, one of the above should unambiguously identify the element.

MWG protocol

* approximate lengths if exact sequence not known

* gene name and links to appropriate databases

e.g., SWISS-PROT, or organism specific databases), if known and relevant

(Normally this information will be provided in one or more spread-sheets or tab-delimited files.)

3. Samples: samples used, extract preparation and labeling

By a 'sample' we understand the biological material, from which the RNA gene products (or DNA) have been extracted for subsequent labeling, hybridisation and measuring. This section describes the source of the sample (e.g., organism, cell type or line), its treatment, as well as preparing the extract and its labeling, i.e., all steps that precedes the contact with an array (i.e., hybridisation). Each sample used in the experiment has a separate section 3. In practice, if the treatments are similar, differing only slightly, the descriptions can be given together, clearly pointing out the differences.
sample source and treatment (this section describes the biological treatment which happens before the extract preparation and labelling, i.e., biological sample in which we intend to measure the gene expression; for each sample only some of the qualifiers given below may be relevant):

* ID as used in section 1

wild type, mutant

* organism (NCBI taxonomy)

Escherichia coli

additional "qualifier, value, source" list; each qualifier in the list is obligatory if applicable; the list includes:

* cell source and type (if derived from primary sources (s))

strain BW25113
(lacI^{q} *rrnB*$_{T14}$ Δ*lacZ*$_{WJ16}$ *hsdR514* Δ*araBAD*$_{AH33}$ Δ*rhaBAD*$_{LD78}$) derivat of F^{-} λ^{-} K12 strain BD792

* development stage

* genetic variation (e.g., gene knockout, transgenic variation)

mutant = gene knockout: *ΔrpoE*

* in vivo treatments (organism or individual treatments)

* in vitro treatments (cell culture conditions)

cells were grown at 37°C in Mineral salts medium + 0.2 % glycerol + 0.3 % casaminoacids

* treatment type (e.g., small molecule, heat shock, cold shock, food deprivation)

metals

* compound

ZnCl2, CuCl2, CdCl2

* separation technique (e.g., none, trimming, microdissection, FACS)

none

* laboratory protocol for sample treatment

At a cell turbidity of 100 Klett the different metals (to a final concentration of 100 µM ZnCl2 or 250 µM CuCl2 or 25 µM CdCl2 respectively) were added and incubation was continued for 10 min. Then cells were harvested.

b) hybridisation extract preparation laboratory protocol for extract preparation, including:

protocol description

* Description:

Total RNA preparation from *Escherichia coli*

Grow *E. coli* culture in medium to a cell turbidity of 100 Klett, add metals,
incubate for 10 min, then harvest cells by centrifugation for 1' in 2.0 ml Tubes.
Quickly freeze in liquid nitrogen.
Resuspend pellet of 10 ml cells in 0.5 ml buffer AE and transfer immediately to
a mixture at 60 °C (6 ml lysisbuffer, 72 µl 20% SDS, 2.5 ml buffer AE) and mix.
Incubate at 60 °C for 5 min with shaking, than incubate for 10 min in NaCl-ice-mix.
Centrifuge for 20 min at 10 °C and transfer upper phase to 3 ml lysisbuffer, 300 µl sodium
acetate. Mix well, centrifuge for 10 min at 4 °C, repeat this step three times.
Precipitate with 2.5 volume of Ethanol at -20 °C, centrifuge 1 h at 4 °C and resuspend
the pellet in 100 µl of DEPC-H_2O.

Materials:
Buffer AE:
20 mM sodium acetate, pH = 5.5, 1 mM EDTA
lysis buffer:
1 volume of phenol pH 5.2, 1 volume of buffer AE,
0.1 % SDS, 1 volume chloroform

* extraction method

hot phenol

* whether total RNA, mRNA, or genomic DNA is extracted

total RNA

* amplification (RNA polymerases, PCR)

none

* optional "qualifier, value, source" list (see Introduction)

c)labelling: laboratory protocol for labelling, including:

* protocol

MWG labeling protocol was used (with some modifications).
cDNA synthesis was primed with hexamers.

* amount of nucleic acids labeled

50 μg total RNA

* label used (e.g., Cy3, Cy5, 33P)

Cy3, Cy5

* optional "qualifier, value, source" list (see Introduction)

4. Hybridisations: procedures and parameters

This section describes details of each hybridisation in the experiment.
Each hybridisation has a separate section 4, though if they are similar they may be described together.

* ID as given in section 1

H1-H16

* laboratory protocol for hybridisation, including:

* the solution (e.g., concentration of solutes)

Hybridisation buffer:
Salt based buffer (MWG)

* blocking agent

none

* Slide blocking:

none

* Probe blocking:

none

* wash procedure

5 min wash with 2x SSC, 0.1% SDS
5 min wash with 1x SSC
5 min wash with 0.1x SSC

* quantity of labelled target used

equal amounts of cDNA

* time, concentration, volume, temperature

overnight (20h), 120 µl, at 42°C

* description of the hybridisation instruments

Corning Hybridisation chamber in shaking waterbath

* optional "qualifier, value, source" list (see Introduction)

5. Measurements: images, quantitation, specifications:
This section describes the data obtained from each scan and their combinations
hybridisation scan raw data:

a1) the scanner image file (e.g., TIFF) from the hybridised microarray scanning
attached files

available on request

ii.a2) scanning information:

* parsed header of the TIFF file, including laser power, spatial resolution, pixel space, PMT voltage;

available on request

* laboratory protocol for scanning, including:

* hybridisation ID as in Section 1

H1-H16

* image unique id

* scanning parameters (including laser power,spatial resolution,pixel space PMT voltage)

* lab protocol for scanning (including scanning hardware and software)

* scanning hardware

Affymetrix Array Scanner 428

* scanning software

Array Scanner software Version 1.1.0.100.

b) image analysis and quantitation
bi) the complete image analysis output (of the particular image analysis software) for each element (or composite element - see 2.b)),
for each channel -

see attached files:

available on request

bii) image analysis information:

* input image id

* quantitation unique id

* image analysis software specification and version, availability, and the description of the algorithm

ImaGene V4.2, (Biodiscovery Inc. El Segundo, CA)

* all parameters

Expected spot diameter: 9-22 Micrometer

pixel intensity:	35-85
Background intensity	10-89
Background buffer	3.0
Background width	3.0
Empty spots	2
Poor spots	0.5

C) summarized information from possible replicates

ci) derived measurement value summarizing related elements as used by the author (this may constitute replicates of the element on the same or different arrays or hybridisations, as well as different elements related to the same entity e.g. gene)

cii) reliability indicator for the value of c1) as used by the author (e.g. standard deviation); may be "unknown"

ciii) specification how c1 and c2 are calculated; the specification should be bases on b1

6. Normalisation controls, values, specifications for hybridisations

a) Normalization strategy (spiking, housekeeping genes, total array)

total array

b) Normalisation algorithm (linear regression, log-linear regression, ratio statistics, log(ratio) mean median centering)

linear regression

c) Control array elements

* position (the abstract coordinate on the array)

available on request

* control type (spiking, normalization, negative, positive)

negative

* control qualifier (endogenous, exogenous)

exogenous

d) Hybridisation extract preparation

* spike type

none

* target element

* optional user defined quality value
(to be added as section 7 in the next MIAME version)

The ten comparisons of the microarray data are provided as the Microsoft Excel file SuppMat.xls